

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Satoko YAMAHIRA et al.

Group Art Unit: 1651

Application No. 10/568,671

Examiner: MARX, IRENE.

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Confirmation No.: 2722

For: LACTIC ACID BACTERIA  
CAPABLE OF STIMULATING MUCOSAL  
IMMUNITY

Commissioner for Patents  
PO Box 1450  
Alexandria, VA 22313-1450

Sir:

DECLARATION UNDER 37 C.F.R. § 1.132

I, Masamichi TOBA, do hereby make the following  
declaration:

1. I am one of the named inventors of the  
above-identified application, and am familiar with the subject  
matter of said application as well as the disclosures in the  
cited references.

2. In order to demonstrate the advantages of the  
present invention, the following experiments were carried out  
under my direction and supervision.

## Experimental Report

### I. Purpose

This experiment is intended to examine the influence of the intake of the *Lactobacillus plantarum* ONRIC b0240 strain (i.e., *Lactobacillus pentosus* ONRIC b0240 (FERM BP-10065); hereinafter referred to as "b0240") of lactic acid bacteria on the total amounts of IgA in the saliva of healthy adult women.

### II. Materials and Methods

The present study involved 30 healthy adult females (age: 21 to 24 years old) to examine the influence of the intake of b0240-containing drinking water on the salivary total IgA amount.

The subjects were divided into three groups: a water intake group (control), an b0240 ( $1 \times 10^7$  CFU/ml) intake group, and an b0240 ( $1 \times 10^8$  CFU/ml) intake group.

More specifically, the study was conducted as follows: the placebo (b0240-free drinking water) intake group was a control group, and the b0240 intake groups ingested 200ml of drinking water containing dead cells of b0240, which were prepared by heating a lactic acid concentrated solution (b0240:  $10^{10}$  CFU/ml) at 140°C for 5 seconds, in an amount of  $1 \times 10^7$  CFU/ml or  $1 \times 10^8$  CFU/ml for three weeks. Before and after the study period (day 0 and day 21), saliva was collected, and the total amount of IgA in the salivary samples were measured. Salivary samples were also collected on day 10 to measure the salivary total IgA amount. The salivary samples were obtained using Salivette for one minute. Each parameter was measured by the ELISA method.

The salivary total IgA amount was determined by calculating the variations (differences) between the pre-treatment value (day 0) and treatment value (day 10) and between the pre-treatment value (day 0) and post-treatment value (day 21) of each subject. Each of the obtained variations was evaluated by the two-tailed Dunnett's test for comparison with the control group. The significance level was 5%.

Excluding 4 subjects who took medications during the treatment period, 26 subjects were evaluable for the efficacy analysis.

### III. Results and Consideration

The increments of the salivary total IgA amount during the treatment period were as follows. After the 10<sup>th</sup> day of intake, the  $1 \times 10^7$  CFU/ml intake group ( $24.0 \pm 31.7$   $\mu$ g) and the  $1 \times 10^8$  CFU/ml intake group ( $37.7 \pm 37.7$   $\mu$ g) showed higher values than the water intake group ( $19.2 \pm 51.5$   $\mu$ g). Further, after the 21<sup>st</sup> day of intake, the  $1 \times 10^7$  CFU/ml intake group ( $52.2 \pm 24.4$   $\mu$ g) and the  $1 \times 10^8$  CFU/ml intake group ( $15.6 \pm 34.1$   $\mu$ g) also showed higher values than the placebo group ( $-21.8 \pm 28.3$   $\mu$ g). These results were statistically significant (Table 1).

As is clear from the results, the salivary total IgA amount in healthy adults was increased by the intake of b0240. This suggests that the b0240 of lactic acid bacteria have the potential to contribute to the body's defense, such as protection against infection and relief of allergic symptoms, by enhancing the mucosal immune function.

Table 1

The amount of total IgA in salivary samples

Salivary IgA in supplemented b0240 and placebo groups.

Data value are the mean  $\pm$  SD. \*\*P<0.01 compares with the placebo group.

Treatment	Amount ( $\mu$ g)			Amount of increase ( $\mu$ g)	
	Day 0	Day 10	Day 21	Day 0-Day 10	Day 0-Day 21
placebo	87.0 $\pm$ 42.4	106.1 $\pm$ 58.8	66.0 $\pm$ 38.2	19.2 $\pm$ 51.5	-21.8 $\pm$ 28.3
b0240 ( $1 \times 10^7$ /ml)	43.9 $\pm$ 31.8	67.9 $\pm$ 20.3	96.1 $\pm$ 38.4	24.0 $\pm$ 31.7	52.2 $\pm$ 24.4**
b0240 ( $1 \times 10^8$ /ml)	53.5 $\pm$ 38.4	91.2 $\pm$ 47.4	69.1 $\pm$ 35.2	37.7 $\pm$ 37.7	15.6 $\pm$ 34.1**

I, the undersigned, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 20 May 2010

By



Masamichi TOBA